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PREPARATION OF DOUGH AND BAKED PRODUCTS

FIELD OF THE INVENTION

The invention relates to the retardation of staling of baked products during storage.

BACKGROUND OF THE INVENTION

Staling of baked products (such as bread) has been recognized as a problem which becomes more serious as more time lies between the moment of preparation of the bread product and the moment of consumption. The term staling is used to describe changes undesirable to the consumer in the properties of the bread product after leaving the oven, such as an increase of the firmness of the crumb, a decrease of the elasticity of the crumb, and changes in the crust, which becomes tough and leathery.

The firmness of the bread crumb increases further during storage up to a level, which is considered as negative. The increase in crumb firmness, which is considered as the most important aspect of staling, is recognized by the consumer a long time before the bread product has otherwise become unsuitable for consumption.

Enzymatic retardation of staling by means of various endo-amylases has been described. Thus, US 2,615,810; US 3,026,205 and O. Silberstein, "Heat-Stable Bacterial Alpha-Amylase in Baking", Baker's Digest 38(4), Aug. 1964, pp. 66-70 and 72, describe the use of alpha-amylase. WO 91/04669 (Novo Nordisk) describes the use of a maltogenic endo-amylase from *Bacillus stearothermophilus.*

It is also known to add a phospholipase to dough. Thus, US 4,567,046 (Kyowa Hakko) discloses that the addition of phospholipase A substantially free from lipase and protease enhances the properties of the dough and of bread made from the dough, including retardation of the staling.

M.R. Kweon et al., Journal of Food Science, 59 (5), 1072-1076 (1994) disclose the effect of 2-4 % by weight of phospholipid hydrolysate together with an antistaling amylase on the retrogradation of starch in bread.

SUMMARY OF THE INVENTION

The invention provides a process for preparing a dough or a baked product prepared from the dough which comprises adding to the dough an endo-amylase, a phospholipase and a phospholipid. The invention also provides a dough and a pre-mix comprising these ingredients.

Compared to a control without enzyme addition, the addition of endo-amylase according to the prior art increases the initial firmness of a baked product, but retards the crumb firming during storage. The addition of phospholipid + phospholipase according to the invention is effective in avoiding the increased initial firmness and in further reducing the rate of crumb firming during storage, compared to the endo-

amylase alone. The process of the invention avoids any significant change in the taste or smell of the baked product.

DETAILED DESCRIPTION OF THE INVENTION

Endo-amylase

The endo-amylase used in the invention may be any endo-amylase that is effective in retarding the staling (crumb firming) of baked products.

The amylase preferably has a temperature optimum in the presence of starch in the range of 30-90°C, preferably 50-80°C, particularly 55-75°C, e.g. 60-70°C. The temperature optimum may be measured in a 1 % solution of soluble starch at pH 5.5.

A preferred example is a maltogenic endo-amylase from *Bacillus stearothermophilus*, commercially available from Novo Nordisk A/S under the tradename Novamyl®. This is further described in US 4,598,048 and US 4,604,355.

Other examples of endo-amylases are fungal and bacterial alpha-amylases, derived e.g. from *Aspergillus*, particularly *A. oryzae*, or from *Bacillus*, particularly *B. licheniformis* or *B. amyloliquefaciens*.

The endo-amylase is added in an effective amount for retarding the staling (crumb firming) of the baked product. The amount of endo-amylase will typically be in the range of 0.01-10 mg of enzyme protein per kg of flour, e.g. 1-10 mg/kg. A maltogenic endo-amylase is preferably added in an amount of 50-5000 MANU/kg of flour, e.g. 100-1000 MANU/kg. One MANU (Maltogenic Amylase Novo Unit) may be defined as the amount of enzyme required to release one µmol of maltose per minute at a concentration of 10 mg of maltotriose (Sigma M 8378) substrate per ml of 0.1 M citrate buffer, pH 5.0 at 37 °C for 30 minutes.

Phospholipid

The phospholipid is a diacyl-glycero-phospholipid, e.g. lecithin or cephalin. It is preferably added in an amount of 0.5-50 g/kg of flour, e.g. 1-10 g/kg.

Phospholipase

The phospholipase may have A_1 or A_2 activity to remove fatty acid from the phospholipid and form a lyso-phospholipid. It may or may not have lipase activity, i.e. activity on triglycerides. The phospholipase preferably has a temperature optimum in the range of 30-90°C, e.g. 30-70°C.

The phospholipase may be of animal origin, e.g. from pancreas (e.g. bovine or porcine pancreas), snake venom or bee venom. Alternatively, the phospholipase may be of microbial origin, e.g. from filamentous fungi, yeast or bacteria, such as the genus or species *Aspergillus, A. niger, Dictyostelium, D. discoideum, Mucor, M. javanicus, M. mucedo, M. subtilissimus, Neurospora, N. crassa, Rhizomucor, R. pusillus, Rhizopus, R. arrhizus, R. japonicus, R. stolonifer, Sclerotinia, S. libertiana, Trichophyton, T. ru-*

brum, Whetzelinia, W. sclerotiorum, Bacillus, B. megaterium, B. subtilis, Citrobacter, C. freundii, Enterobacter, E. aerogenes, E. cloacae Edwardsiella, E. tarda, Erwinia, E. herbicola, Escherichia, E. coli, Klebsiella, K. pneumoniae, Proteus, P. vulgaris, Providencia, P. stuartii, Salmonella, S. typhimurium, Serratia, S. liquefasciens, S. marcescens, Shigella, S. flexneri, Streptomyces, S. violeceoruber, Yersinia, or *Y. enterocolitica.* A preferred phospholipase is derived from a strain of *Fusarium*, particularly *F. oxysporum*, e.g. from strain DSM 2672, as described in co-pending PCT/DK 97/00557.

The phospholipase is added in an amount which at least partly hydrolyzes the phospholipid during the baking process. The amount of phospholipase will typically be in the range of 0.01-10 mg of enzyme protein per kg of flour, e.g. 1-10 mg/kg. A phospholipase with lipase activity is preferably added in an amount corresponding to an lipase activity of 20-1000 LU/kg of flour, particularly 50-500 LU/kg. One LU (Lipase Unit) is defined as the amount of enzyme required to release 1 µmol butyric acid per minute at 30.0°C; pH 7.0; with Gum Arabic as emulsifier and tributyrin as substrate.

Dough

The dough of the invention generally comprises wheat meal or wheat flour and/or other types of meal, flour or starch such as corn flour, corn starch, rye meal, rye flour, oat flour, oat meal, soy flour, sorghum meal, sorghum flour, potato meal, potato flour or potato starch.

The dough of the invention may be fresh, frozen or par-baked.

The dough of the invention is normally a leavened dough or a dough to be subjected to leavening. The dough may be leavened in various ways, such as by adding chemical leavening agents, e.g., sodium bicarbonate or by adding a leaven (fermenting dough), but it is preferred to leaven the dough by adding a suitable yeast culture, such as a culture of *Saccharomyces cerevisiae* (baker's yeast), e.g. a commercially available strain of *S. cerevisiae*.

The dough may also comprise other conventional dough ingredients, e.g.: proteins, such as milk powder, gluten, and soy; eggs (either whole eggs, egg yolks or egg whites); an oxidant such as ascorbic acid, potassium bromate, potassium iodate, azodicarbonamide (ADA) or ammonium persulfate; an amino acid such as L-cysteine; a sugar; a salt such as sodium chloride, calcium acetate, sodium sulfate or calcium sulfate.

The dough may comprise fat such as granulated fat or shortening, but the invention is particularly applicable to a dough where less than 1 % by weight of fat (triglyceride) is added, and particularly to a dough which is essentially fat-free.

The dough may further comprise an emulsifier such as mono- or diglycerides, diacetyl tartaric acid esters of mono- or diglycerides, sugar esters of fatty acids, polyglycerol esters of fatty acids, lactic acid esters of monoglycerides, acetic acid esters of monoglycerides, polyoxyethylene stearates, or lysolecithin, but the invention is par-

ticularly applicable to a dough which is essentially free from emulsifiers other than the phospholipid.

Additional enzyme

Optionally, an additional enzyme may be used together with the endo-amylase and the phospholipase. The additional enzyme may be a second amylase, such as an amyloglucosidase, a beta-amylase, a cyclodextrin glucanotransferase, or the additional enzyme may be a peptidase, in particular an exopeptidase, a transglutaminase, a lipase, a cellulase, a hemicellulase, in particular a pentosanase such as xylanase, a protease, a protein disulfide isomerase, e.g., a protein disulfide isomerase as disclosed in WO 95/00636, a glycosyltransferase, a branching enzyme (1,4-α-glucan branching enzyme), a 4-α-glucanotransferase (dextrin glycosyltransferase) or an oxidoreductase, e.g., a peroxidase, a laccase, a glucose oxidase, a pyranose oxidase, a lipoxygenase, an L-amino acid oxidase or a carbohydrate oxidase.

The additional enzyme may be of any origin, including mammalian and plant, and preferably of microbial (bacterial, yeast or fungal) origin and may be obtained by techniques conventionally used in the art.

The xylanase is preferably of microbial origin, e.g. derived from a bacterium or fungus, such as a strain of *Aspergillus*, in particular of *A. aculeatus*, *A. niger* (cf. WO 91/19782), *A. awamori* (WO 91/18977), or *A. tubigensis* (WO 92/01793), from a strain of *Trichoderma*, e.g. *T. reesei*, or from a strain of *Humicola*, e.g. *H. insolens* (WO 92/17573, the contents of which is hereby incorporated by reference). Pentopan® and Novozym 384® (both from Novo Nordisk A/S) are commercially available xylanase preparations produced by *Trichoderma reesei*.

The amyloglucosidase may be an *A. niger* amyloglucosidase (such as AMG™, available from Novo Nordisk A/S, Denmark). Other useful amylase products include Grindamyl® A 1000 or A 5000 (available from Grindsted Products, Denmark) and Amylase® H or Amylase® P (available from Gist-Brocades, The Netherlands).

The glucose oxidase may be a fungal glucose oxidase, in particular an *Aspergillus niger* glucose oxidase (such as Gluzyme®, available from Novo Nordisk A/S, Denmark).

The protease may in particular be Neutrase® (available from Novo Nordisk A/S, Denmark).

The lipase may be derived from a strain of *Thermomyces* (*Humicola*), *Rhizomucor*, *Candida*, *Aspergillus*, *Rhizopus*, or *Pseudomonas*, in particular from *Thermomyces lanuginosus* (*Humicola lanuginosa*), *Rhizomucor miehei*, *Candida antarctica*, *Aspergillus niger*, *Rhizopus delemar* or *Rhizopus arrhizus* or *Pseudomonas cepacia*. In specific embodiments, the lipase may be Lipase A or Lipase B derived from *Candida antarctica* as described in WO 88/02775, or the lipase may be derived from *Rhizomucor miehei* as described in EP 238,023, or *Humicola lanuginosa* de-

scribed in EP 305,216, or *Pseudomonas cepacia* as described in EP 214,761 and WO 89/01032.

Baked product

The process of the invention may be used for any kind of baked product prepared from dough, either of a soft or a crisp character, either of a white, light or dark type. Examples are bread (in particular white, whole-meal or rye bread), typically in the form of loaves or rolls, French baguette-type bread, pita bread, tortillas, cakes, pancakes, biscuits, cookies, pie crusts, crisp bread, steamed bread, pizza and the like.

Pre-mix

The present invention further relates to a pre-mix comprising flour together with an endo-amylase, a phospholipase and a phospholipid. The pre-mix may contain other dough-improving and/or bread-improving additives, e.g. any of the additives, including enzymes, mentioned above.

Enzyme preparation

The invention provides an enzyme preparation comprising an endo-amylase and a phospholipase, for use as a baking additive in the process of the invention. The enzyme preparation is preferably in the form of a granulate or agglomerated powder. It preferably has a narrow particle size distribution with more than 95 % (by weight) of the particles in the range from 25 to 500 μm.

Granulates and agglomerated powders may be prepared by conventional methods, e.g. by spraying the amylase onto a carrier in a fluid-bed granulator. The carrier may consist of particulate cores having a suitable particle size. The carrier may be soluble or insoluble, e.g. a salt (such as NaCl or sodium sulfate), a sugar (such as sucrose or lactose), a sugar alcohol (such as sorbitol), starch, rice, corn grits, or soy.

EXAMPLES

Example 1

Bread was baked with phospholipase from *Fusarium oxysporum*, maltogenic endo-amylase from *B. stearothermophilus* (Novamyl) and phospholipid (lecithin). The dosages were 750 MANU/kg of the endo-amylase and 10 g/kg of the phospholipid; the dosage of phospholipase is indicated below. As reference, bread was also baked without one or more of these ingredients.

Doughs were prepared according to the standard European straight dough procedure (ABF-SP-1201.01/01), except that 50 g yeast was added per kg of flour (instead of 40 g/kg) and 40 ppm of ascorbic acid was added. The doughs were scaled to 2350 g and baked in lidded pans.

The crumb firmness was measured using a texture analyzer TA-XT2 from Stable Micro Systems. Texture was measured according to a modified ACCA method 74-09 described in Standard Operation Procedure ABF-SM-1502.01/01. These measurements were made after 0 days (approximately 2 hours after baking) and again after 1, 2 and 7 days storage (wrapped in double plastic bags and stored at 22°C).

The results are shown as firmness versus additive and storage time:

Additives	Phospholipase dosage (LU/kg)	Day 0	Day 1	Day 2	Day 7
Invention: Endo-amylase + phospholipase + phospholipid	50	316	417	517	868
	250	279	371	455	790
	500	248	324	410	752
Reference:					
None (control)	0	296	875	1207	2162
Endo-amylase	0	469	563	801	1083
Phospholipid + phospholipase	50	208	470	782	1560
	250	231	467	721	1424
	500	233	420	649	1303

The results show that compared to the control, the addition of endo-amylase increases the initial firmness, but retards the crumb firming during storage. The addition of phospholipid + phospholipase according to the invention is effective in avoiding the increased initial firmness and further reducing the rate of crumb firming during storage, compared to the endo-amylase alone.

CLAIMS

1. A process for preparing a dough or a baked product prepared from the dough which comprises adding to the dough an endo-amylase, a phospholipase and a phospholipid.

2. The process of the preceding claim wherein the endo-amylase has a temperature optimum in bread at 70-90°C.

3. The process of either preceding claim wherein the endo-amylase is from *Bacillus*, and is preferably a maltogenic amylase from *B. stearothermophilus*.

4. The process of any preceding claim wherein the phospholipase has a temperature optimum of 30-70°C.

5. The process of any preceding claim wherein the phospholipase is fungal, preferably from *Fusarium*, most preferably from *F. oxysporum*.

6. The process of any preceding claim wherein the phospholipid comprises lecithin.

7. The process of any preceding claim wherein the dough is essentially fat-free.

8. The process of any preceding claim wherein the dough is essentially free of lysophospholipid.

9. The process of any preceding claim wherein the dough is essentially free of emulsifiers other than the phospholipid.

10. The process of any preceding claim wherein the dough consists essentially of flour, water, yeast, salt and sugar.

11. A dough which comprises an endo-amylase, a phospholipase and a phospholipid.

12. A pre-mix for dough comprising an endo-amylase, a phospholipase and a phospholipid.

13. An enzyme preparation which comprises an endo-amylase and a phospholipase.

14. The preparation of the preceding claim which further comprises a hemicellulase, preferably a pentosanase, more preferably a xylanase.

15. The preparation of claim 13 or 14 which is a granulate or an agglomerated powder.

16. The additive of any of claims 13-15 wherein more than 95 % (by weight) has a particle size between 25 and 500 μm.